A study on the relationship between egg number and density in tipula subnodicornis zetterstedt

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A Study on the relationship between egg number and density in *Tipula subnodicornis* Zetterstedt

A dissertation submitted by
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to the University of Durham for the
degree of Master of Science

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Contents

INTRODUCTION

This study was based on Coulson's work (1956,1959 and 1962) on Tipula subnodicornis Zetterstedt carried out between 1953 and 1956 on the Moor House National Nature Reserve.

It was proposed to investigate the relationship between the number of eggs produced by females on an area and the density of larvae or adults on that area. The females of T. subnodicornis are subapterous and do not move far from their place of emergence and the flight of the males is not strong (Coulson 1956) so that the density of adults on a site relates to the larval density.

It was also hoped to compare the fecundity of females on sites with different vegetation. Coulson (1962) showed that larval density varied considerably from one type of vegetation to another. Unfortunately the time consumed in catching the adult insects was such that only a few sites could be used.

It has been shown in a number of insects that factors such as temperature, nutrition, impregnation, the secretion of hormones and the onset of diapause (Wigglesworth 1965) affect the number and fertility of the eggs produced by the female. In many species the eggs mature in the imago so these effects may operate both on the mature and immature stages. In T. subnodicornis the eggs are fully developed when the adult emerges and the majority are layed within 14 hours (Coulson 1956). The adults were never observed feeding. Emergence, copulation and oviposition appeared not to be greatly affected by cold or wet

weather and although emergence was delayed on sunny days (Coulson 1956) this was compensated for by evening activity. It seems unlikely therefore that egg number laid by a female or the fertility of the eggs should be affected at the adult stage. The number of eggs per female, with which the present study was concerned, can only have been affected by factors operating on the immature stages.

As the number of eggs produced by each female was in the hundreds a considerable part of this study was spent in trying to find a correlation between egg numbers and some easily-measured parameter. Such a correlation once established could be used to derive egg numbers for small samples from a great number of different areas on which the larval density had been established. Not only could sites be re-used from year to year but with a greater number of sites it would be easier to assess whether a genuine correlation had been established between density and egg numbers.

THE STUDY AREA

The Moor House National Nature Reserve (N.R. 80 : Nat.Grid Ref. NY/758329) consists of 4,000 hectares lying mainly on the eastern dip slopes of the Pennine escarpment. The three fells, Knock Fell 2,604 ft., Great Dun Fell 2,780 ft. and Little Dun Fell 2,761 ft. which form part of the summit ridge lie within the Reserve.

The climate is severe and has been classified by Manley (1936,1943) as sub-arctic. The rainfall (annual average 70") and the temperature are slightly higher, while the duration of sunlight is possibly somewhat lower at Moor House than it is in the south of Iceland at sea-level.

Much of the Reserve is covered by mixed-moor growing on peat. The peat is frequently cut into and eroded by streams which drain, on the east side of the Reserve, into the River Tees and its tributaries Moss Burn and Trout Beck. Moor House itself stands on a limestone outcrop at a height of 1,840 ft.

THE SITES

The choice of sites was governed partly by their nearness to the house and partly by the affinity of T. subnodicornis for areas where Juncus squarrosus is dominant (Coulson 1956). Preliminary larval sampling carried out from January to early May led to the selection of five sites which are shown on the map (fig.1).

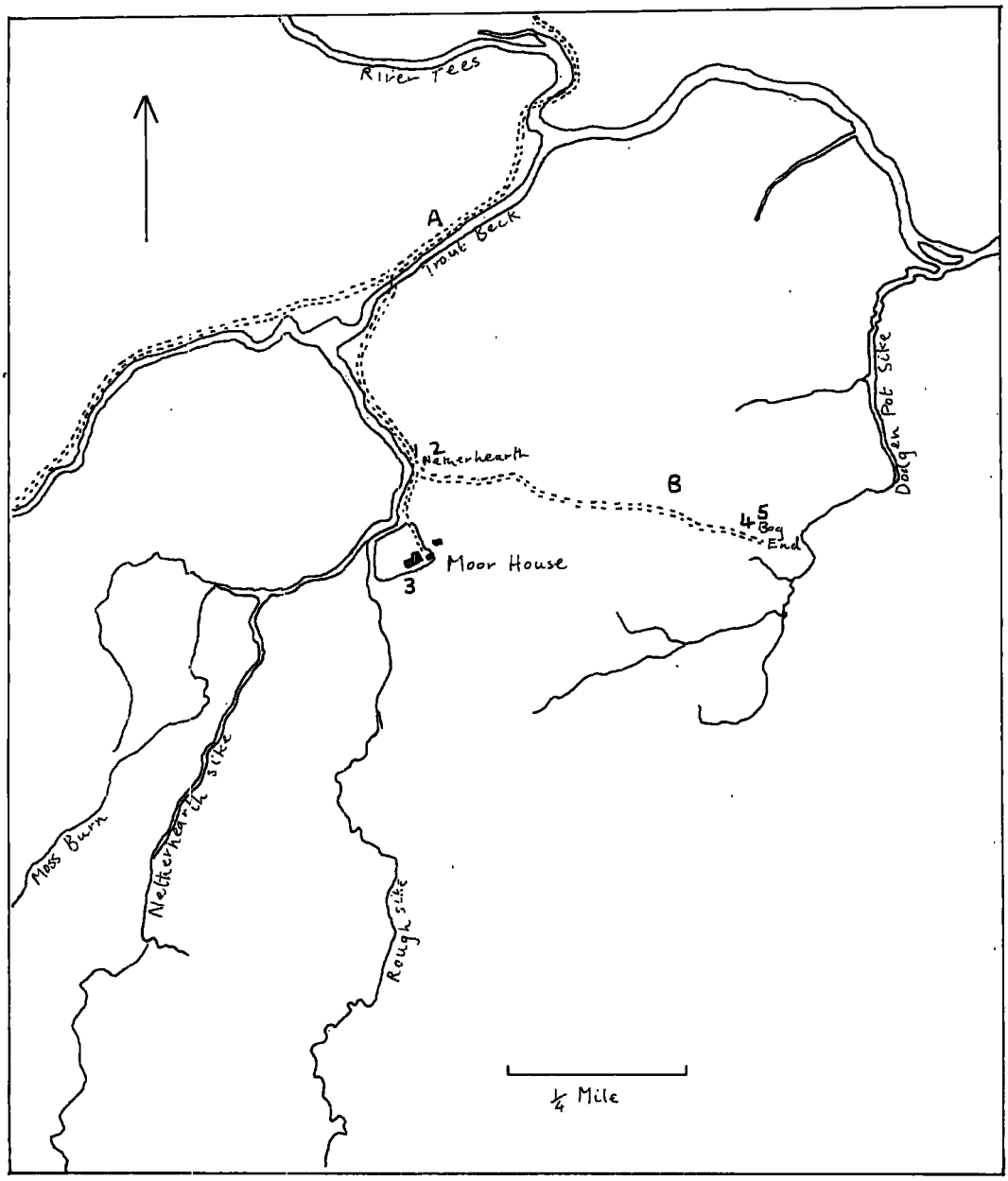


Fig. I A map of part of the Reserve showing the positions of the sites used during this study

Site 1 at Netherhearth measured 15 m. x 15 m. and lay on a flat area where Juncus squarrosus and Festuca ovina form a high proportion of cover. Nardus stricta is common.

Site 2 above Netherheath measured 10 m. x 22.5 m. Its long axis sloped down westwards to Site 1. from which it was separated by a peat hag with a damp area at its base. The vegetation above Netherhearth is dominated by Eriophorum vaginatum and Juncus squarrosus.

Site 3 measured 15 m. x 15 m. and lay on the north facing slope in the pasture behind the house. Juncus squarrosus and Festuca ovina are both widespread. Nardus stricta is also present.

Site 4 measured 45 m. x 5 m. and lay on an old mine-track. The vegetation has been described by Welch (1964) and is dominated by Juncus squarrosus; Deschampsia flexuosa, Carex nigra, Polytrichum commune as well as Festuca ovina are constants. Eriophorum angustifolium is also common. The track is flanked on either side by areas of mixed-moor (Pearsall 1950).

Site 5 measured 15 m. x 15 m. and lay on a west facing slope of mixed-moor close to Site 4. An adjacent area has been described by Welch. Calluna vulgaris and Eriophorum vaginatum have high cover value. Sphagnum plumulosum, Eriophorum angustifolium and Juncus squarrosus are constants.

Two further sites were used to supply extra information.

Site A measured 15 m. x 15 m. and lay on a south-

east facing slope. It was set up as the other five sites with four sticky traps and ten pitfalls. The vegetation of the site has been described (Hadley 1966) and is similar to that of site 4 with the exception that the absence of Eriophorum spp. and the presence of Nardus stricta and a number of other species indicate a drier habitat.

Site B lay to the north of site 5 in an area of similar vegetation and consisted of two rows of twenty pitfalls.

Each site was a rectangle of 225 sq.m. in area. A sticky trap was placed at each of the four corners and two rows of pitfall traps, each trap separated from its neighbours by approximately two metres, were placed within the area.There were 5 pitfalls in each row.

The sticky traps were similar to those described by Broadbent (1948) and consisted of metal cylinders 30 cm. high and 13.7 cm. in diameter, surrounded by polythene coated with "stik-tite". Each cylinder was kept upright by a bamboo stake through its centre.

The pitfalls consisted of 1lb. jam jars sunk with their rims at ground level and provided with about 5 cc. of detergent solution. The detergent acted as a wetting agent which has been shown to increase trap efficiency.

Population Estimation

The density of T. subnodicornis on each site was estimated by four methods : larval sampling, trapping on sticky traps, trapping in pitfalls and catching by hand on each site.

The larval sampling was carried out between January and May when the larvae were in their fourth instar. 40 four-inch in diameter soil cores were taken from each site. Each core was hand-sorted and then checked by extraction in a Berlese funnel.

The pitfalls on each site were emptied once a day, in the morning, from 23 May until 11 June.

The sticky traps on each site were inspected and any T. subnodicornis present removed at intervals during each day from 23 May until 11 June.

Hand catching was carried out on each site with the aim of clearing each area every day. When numbers were low on a site one visit to that site, in the afternoon, was thought sufficient but during the peak of emergence the site was cleared at least twice during the day. All females that were visible and as many males as could be caught were taken and killed by placing in a jar containing cotton wool impregnated with ethyl acetate. This ensured rapid death with little loss of eggs in the female. Females were divided into copulating and non-copulating. Later the insects were preserved in alcohol.

The results from the four sampling methods can be seen in Table 1. It will be noticed that nowhere do two

Table 1. Showing the numbers of larvae taken in soil samples and the numbers of adults caught

Sites	No. of larvae in 40 cores	No. larvae /m^2	Pitfall Nos. ♂	Pitfall Nos. ♀	Total	Sticky trap nos. ♂+♀	Nos. caught by hand ♂	Nos. caught by hand ♀	Total
1	13	39	53	57	110	97	1276	590	1860
2	7	22	34	82	116	128	1484	1004	2488
3	5	15	28	37	65	114	741	566	1307
4	7	22	51	51	102	232	1988	893	2881
5	12	37	29	20	49	184	382	139	521

methods give consistently similar results. This is discussed below.

Evaluation of Estimation Methods

1 Larval sampling

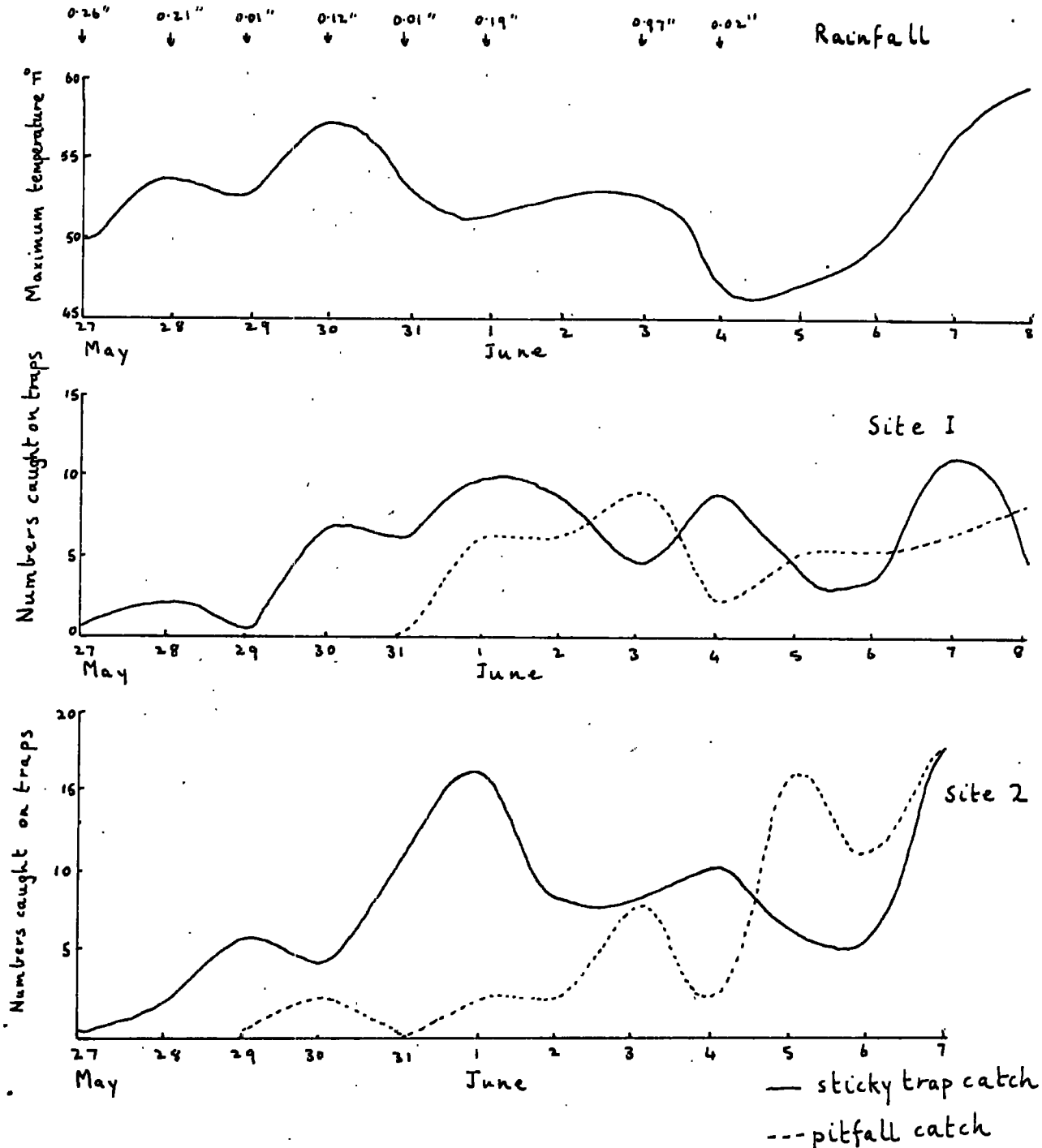
The numbers of larvae/sample were found to be so low that the standard deviation for 40 samples was greater than the mean. Further samples could not be taken after this was discovered as the insects were beginning to pupate ; so it was decided not to use these results in estimations of density.

2 Sticky Trap and Pitfall Estimations

Both sticky traps and pitfalls measure variations in activity as well as variations in population density (Taylor 1963, Greenslade 1964). Taylor has shown that in a number of insects flight ability is little affected by variations in temperature above a minimum temperature but that that temperature is critical in determining whether the insect can fly. From fig. 2 it appears that on 5 and 6 June the temperature was low enough, throughout most of the day, to suppress flying in T. subnodicornis males. The catch of males in the pitfalls was not significantly lowered during this period, but this might be due to an increase in the density of the numbers present rather than a decrease in activity. The rise in the number of females caught in the pitfalls at the same time (table 1) showed that crawling activity continued when flight was not possible.

Coulson (1956) found that on days of prolonged sunshine female emergence and male activity were delayed. It was found during this study that males clustered

Fig. 2 Showing maximum temperature and rainfall from 27 May to 8 June with the daily catch of T. subnodicornis on two sites shown below. ALL readings were taken at 9 a.m.



under the overhang of peat hags when subjected to full sun at mid-day. As long cool evenings followed every day these probably compensated for any decrease in catch due to high mid-day temperatures.

Taylor (1963) cited wind speed as another factor influencing the catches of aphids and other small insects but found that insects above $\frac{1}{4}$" were less affected. Coulson (1956) found that at moderate wind speeds T. subnodicornis flew against the wind. As the wind speed did not exceed 4 (Beaufort) during the study period it can probably be discounted as having any effect on sticky trap catch numbers.

During this study it was found that male flight was suppressed by heavy rain. This is reflected in fig. 2 where the sticky trap catch for 3 June is depressed. This was due to .97" rain falling on 2 June, the only day on which it rained continuously. The pitfall catch shows a corresponding male increase presumably due to an increased density of males on the vegetation.

Greenslade (1964) showed that carabid beetles were affected by the vegetation type on which they were caught, pitfall traps, in a position where there was no obstruction to the horizontal movement of the beetles, being much more successful than those in thick vegetation. Females of T. subnodicornis climbed the nearest vertical surface soon after emergence. On Juncus squarrosus sward this involved some horizontal movement but on the mixed-moor the females were able to crawl upwards in the heather without travelling over the ground. This made them less likely, at this stage, to fall into pitfalls. The males

flew at the level of the Calluna shoots, where the females were hanging, and were also less likely to fall into pitfalls. The fertilised females were most at risk when looking for oviposition sites but even then they would have their horizontal movement impeded. These factors would explain why the proportion of T. subnodicornis caught in the pitfalls to that caught on the sticky traps 49/184 is much lower on site 5, on mixed-moor, than at any of the other sites where the vegetation is of the same physical type and offers less resistance to horizontal movement.

3 Hand Catching

Hand catching is subject to numerous errors but the most obvious difficulty in this study was the comparison of site 5 with the other sites. The difficulty of seeing and catching the flies on site 5 was so great that it cannot be compared with the other sites.

On sites 1-4 the best estimate of comparative density is probably achieved by adding pitfall and sticky trap numbers. It can be seen from fig. 2 that the daily catches by the two methods do to some extent reciprocate each other. When pitfall and sticky trap catches are added the sites may be placed in order of decreasing density (table 2). The order, but not the proportions of the numbers, correspond to those found from hand-catching.

Table 2. Showing numbers caught on traps compared with numbers caught by hand

Site	Pitfall + Sticky trap numbers	Number caught by hand
4	334	2881
2	244	2488
1	207	1860
3	179	1307

Emergence period

In figures 3 and 4 the sticky trap and pitfall catches on 6 sites are shown grouped into three day periods. It is interesting to note that although April and early May were very cold this year the emergence peak, shown by the sticky traps, was only delayed by a three day period. In 1954 the peak occurred from 29 - 31 May and in 1955 from 29 May - 3 June (Coulson 1959). The extended period of the peak, shown by sticky trap figures, on sites 1 and 2 is probably due to the drop in temperature from 4 - 6 June.

Fig. 3 Sticky trap catches of T. subnodicornis on 6 sites (males only)

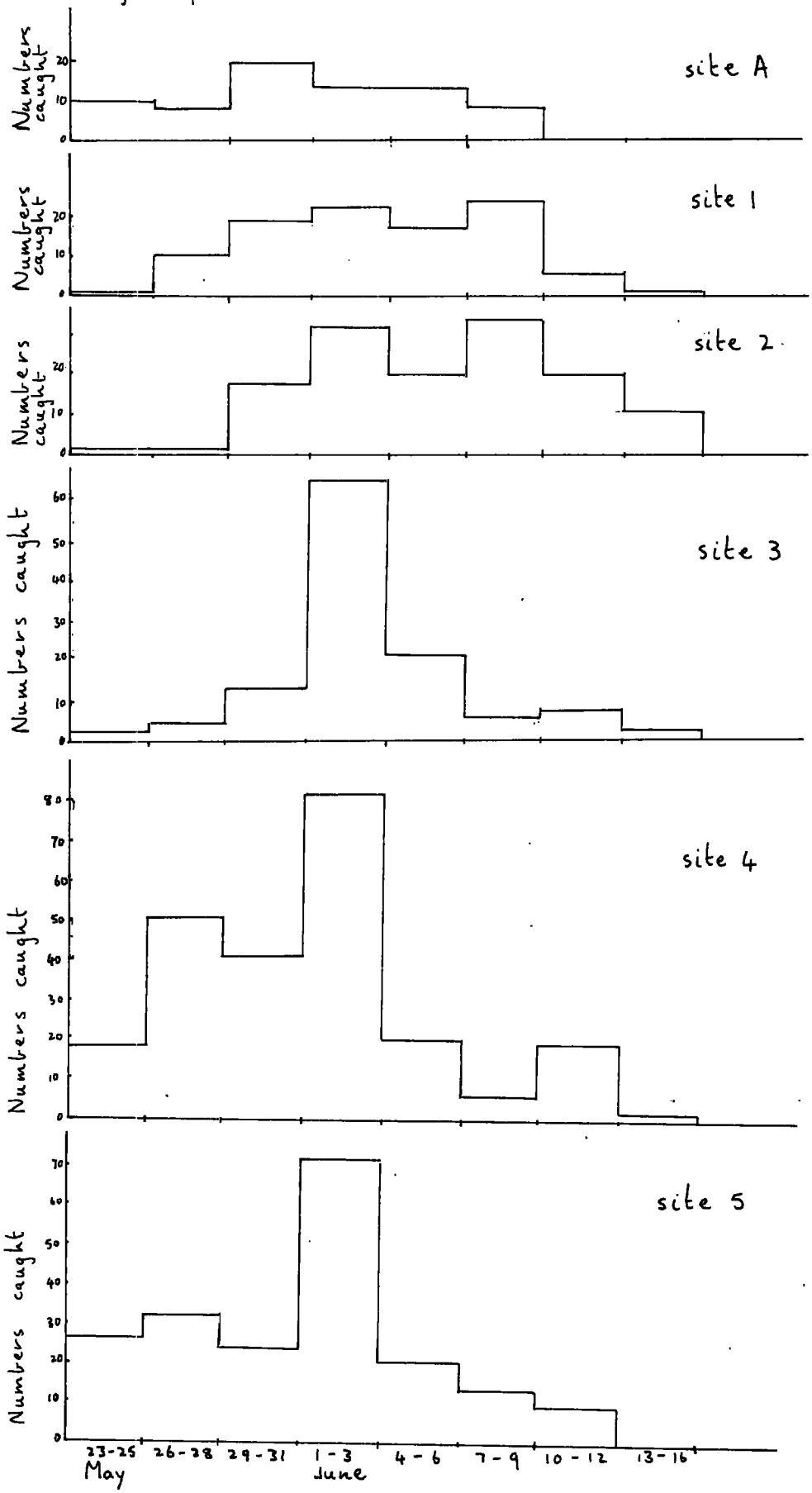
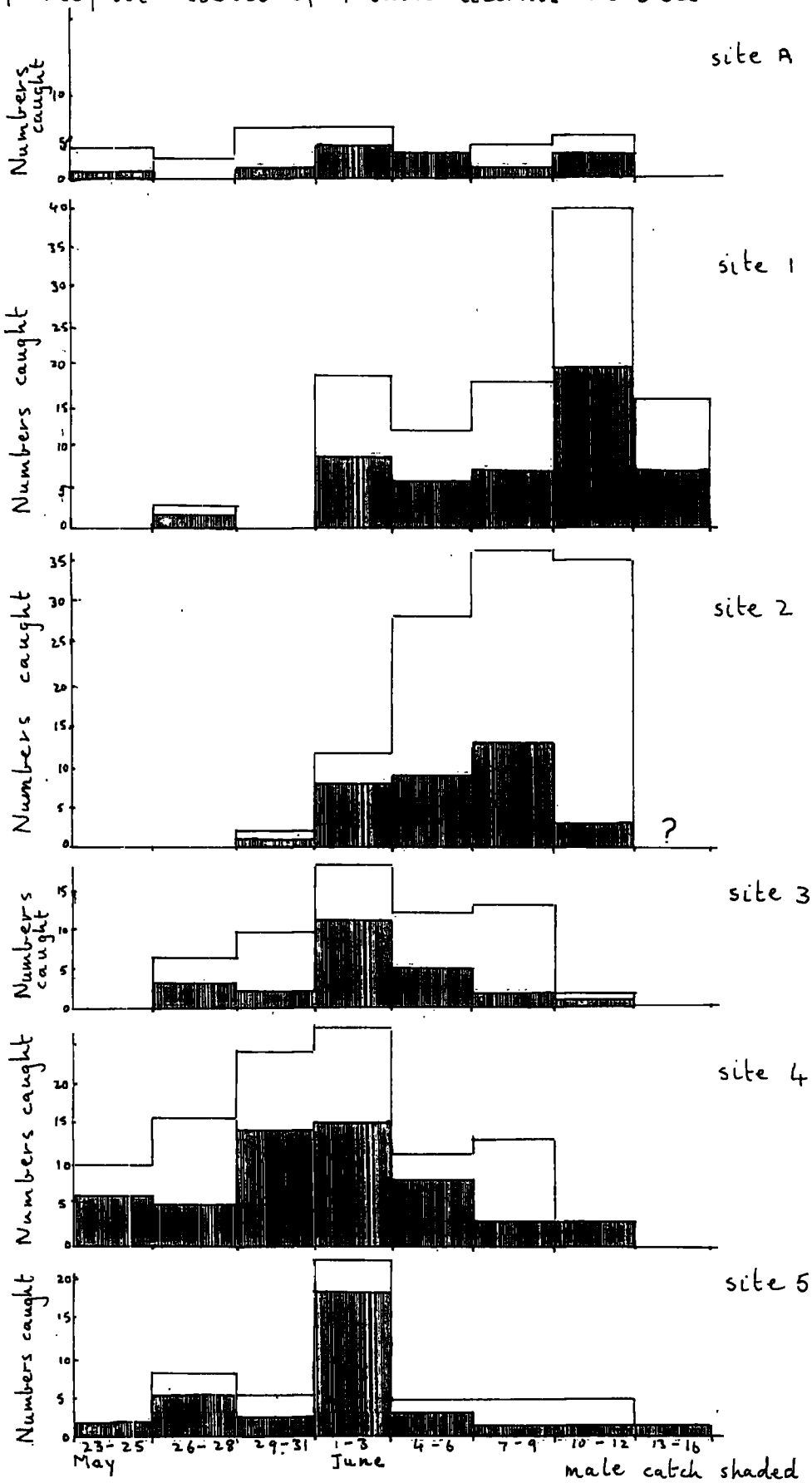


Fig. 4 Pitfall catches of T. subnodicornis on 6 sites



Correlation of egg numbers with an aspect of body size

There were two disadvantages in counting the number of eggs for a sample of females from each site. The number of eggs per female varied from II0 to 480 with a mean of about 300. Counting egg numbers for representative samples from each site would have involved very many hours work. The second disadvantage to egg counting was that only a small percentage of the available females could be used.

During the first days of this study it had been assumed that females of T. subnodicornis taken in copulation would have all their eggs present. It was soon found from dissection that many of the females caught in copulation were spent and retained no eggs. It was also observed in the field that a female would copulate between periods of oviposition.

It was decided to try to find a relationship between female size and the numbers of eggs produced. For this purpose only females known to contain their full number of eggs were used. They were selected by their pale appearance from those caught in copulation. As copulation takes about two hours the cuticle has time to harden and darken during this period. Pale insects caught copulating would not have had time to lay eggs. It was also possible to use non-copulating flies that had been exposed to the air for less than an hour. These were distinguished by their cream colouration and crumpled wings.

Possibly the best relationship between body size and egg numbers would be that between dry weight and egg numbers. This relationship was not used as it could only have been applied to those females retaining their full egg complement or to very large samples. Measurement of the abdomen or whole body length was also rejected. The length of the abdomen decreases during sclerotization and on the laying of eggs.

The wing and leg segment lengths were chosen as measurements that were likely to reflect the size of the body and to be little affected by shrinkage due to hardening of the cuticle or loss of eggs. As the number of eggs present is proportional to the volume of the abdomen a number of correlations using the cube root of the egg number as well as those for egg number were tried.

The eggs from 55 newly emerged females from Site 2 above Netherhearth and from 50 females from Site 4 at Bog End were counted. The wing and tibia lengths were measured for each female on both sites. The tarsus length was also measured on the females from Site 2 but it gave a poor correlation and this measurement was abandoned. The correlation coefficients resulting from the comparison of egg numbers or the cube root of the egg number with the measurements of length are shown in table 3 .

Table 3. Showing the correlation coefficients between egg numbers and body size

Site	Body measurement	egg no.		$\sqrt{\text{egg no.}}$	
		r	p	r	p
2	Tibia			+.49	<.001
55 ♀ measured	Wing	+.504	<.001	+.538	<.001
	Tarsus			+.217	not sig
	Tibia + wing			+.554	<.001
4	Tibia	+.287	<.05		
50 ♀ measured	Wing	+.633	<.001	+.605	<.001
	Wing + tibia	+.65	<.001		

From these results it was decided that the relationships between wing length and egg numbers or the cube root of egg numbers were the most promising. The slight increase in the correlation coefficient when tibia and wing lengths were combined was not considered sufficient to warrant the extra calculation.

Originally the cube root of egg numbers had been chosen on the basis that volume was being compared with length but when the two regressions, egg numbers on wing length, and the cube root of egg numbers on wing length, were compared (figs. 5, 6) it was not clear which was the better. Neither were the two corresponding (y = cube root of egg numbers and y = egg numbers)

Fig. 5 Showing the regression lines for the cube root of egg number plotted against wing length

For site 2 $y = 0.0437x + 2.35$
site 3 $y = 0.062x - 0.58$
site 4 $y = 0.0485x + I.57$

Fig. 6 Showing the regression lines for the egg number plotted against the wing length

For site 2 $y = 5.77x - 264$
site 3 $y = 7.78x - 462$
site 4 $y = 5.82x - 3I3$

√Egg numbers

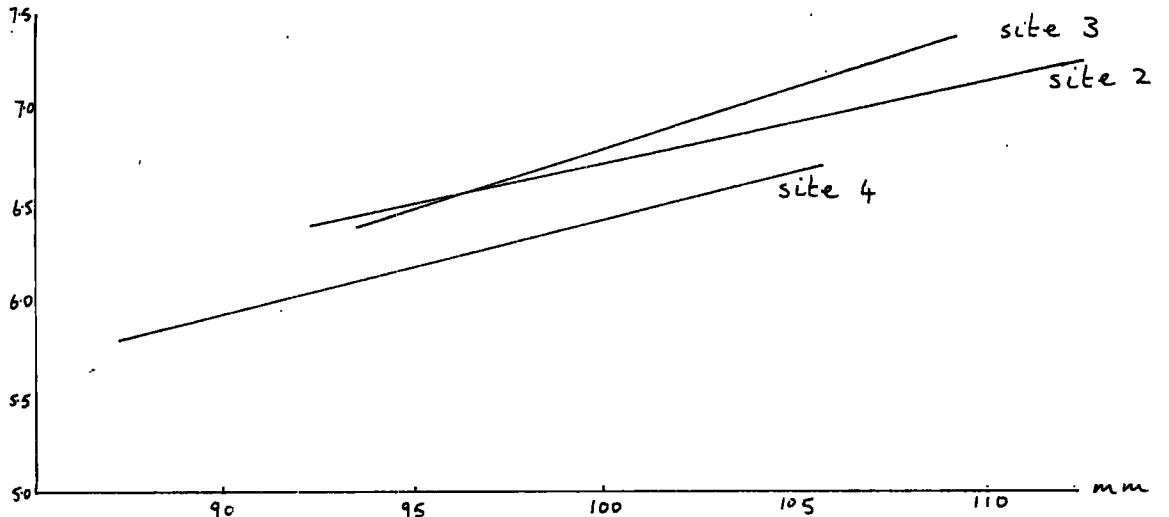


Fig. 5 Wing length

Egg numbers

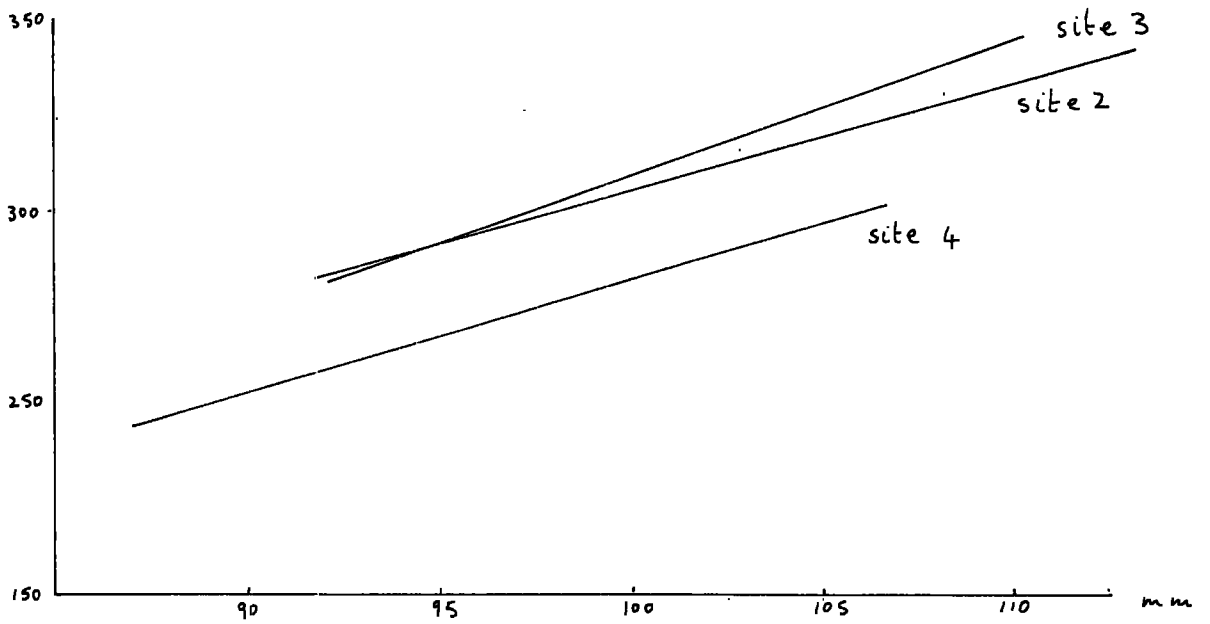


Fig. 6 Wing length

regression lines for each site identical (figs. 5 & 6) so it was decided to count egg numbers from a further site. 36 females from Site 3, behind the house, were dissected, their eggs counted and their wing lengths measured. This provided a further pair of correlation coefficients, shown with those for wing length measurement on Sites 2 and 4 in table 4.

Table 4. Showing correlation coefficients between egg numbers and wing length

Site	Number in sample	Wing x egg number		Wing x cube root of egg number	
		r	p	r	p
2	55	+.504	<.001	+.538	<.001
4	50	+.633	<.001	+.605	<.001
3	36	+.64	<.001	+.72	<.001

The regression lines for this site were again different (figs. 5 & 6) so each pair of regression coefficients was compared (Mather 1949) to see whether the differences were significant. The t values from student's t-test are shown in table 5.

Table 5. Showing comparison of regression coefficients

Sites compared	Egg number		Cube root of egg number	
	t	p	t	p
2 and 4	.68	>.4	.304	>.8
2 and 3	.26	>.7	.813	>.4
3 and 4	.817	>.4	1.46	>.1

As the regression coefficients for the three lines, whether using the cube root of the egg numbers or the egg numbers on the y axis, were not significantly different, the data were pooled using Fisher's Z transformation and two further correlation coefficients calculated. These were as follows :

For combined sites (141 in sample)

Wing length correlated with egg number

$r = +0.587$ $p < .001$

Wing length correlated with cube root of egg number

$r = +0.613$ $p < .001$

The regressions are shown in fig. 7 and fig. 8. For further calculations the relationship between the cube root of the egg number y = 0.092 x -2.59) was used as it had a slightly higher correlation coefficient.

On each site samples of 50, or the number available if below 50, females from each day's catch were taken. The wing length for each female was measured and the mean wing length for each day on each site was calculated. Using these data the mean wing length on each site during the entire emergence period was calculated (table 6).

Table 6. Mean egg numbers derived from the regression y = .092 x -2.59

Site	Mean wing length	Number in sample	SE	Mean egg number /female	SE*
1	101.88	505	±0.45	312	±3.2
2	102.00	565	±0.33	315	±5.3
3	103.8	453	±0.32	340	±3.4
4	96.60	705	±0.35	250	±2.7
5	96.76	131	±1.7	253	±6.7

*The standard error has been calculated on the basis of the regression equation y = (11.05 x -808)

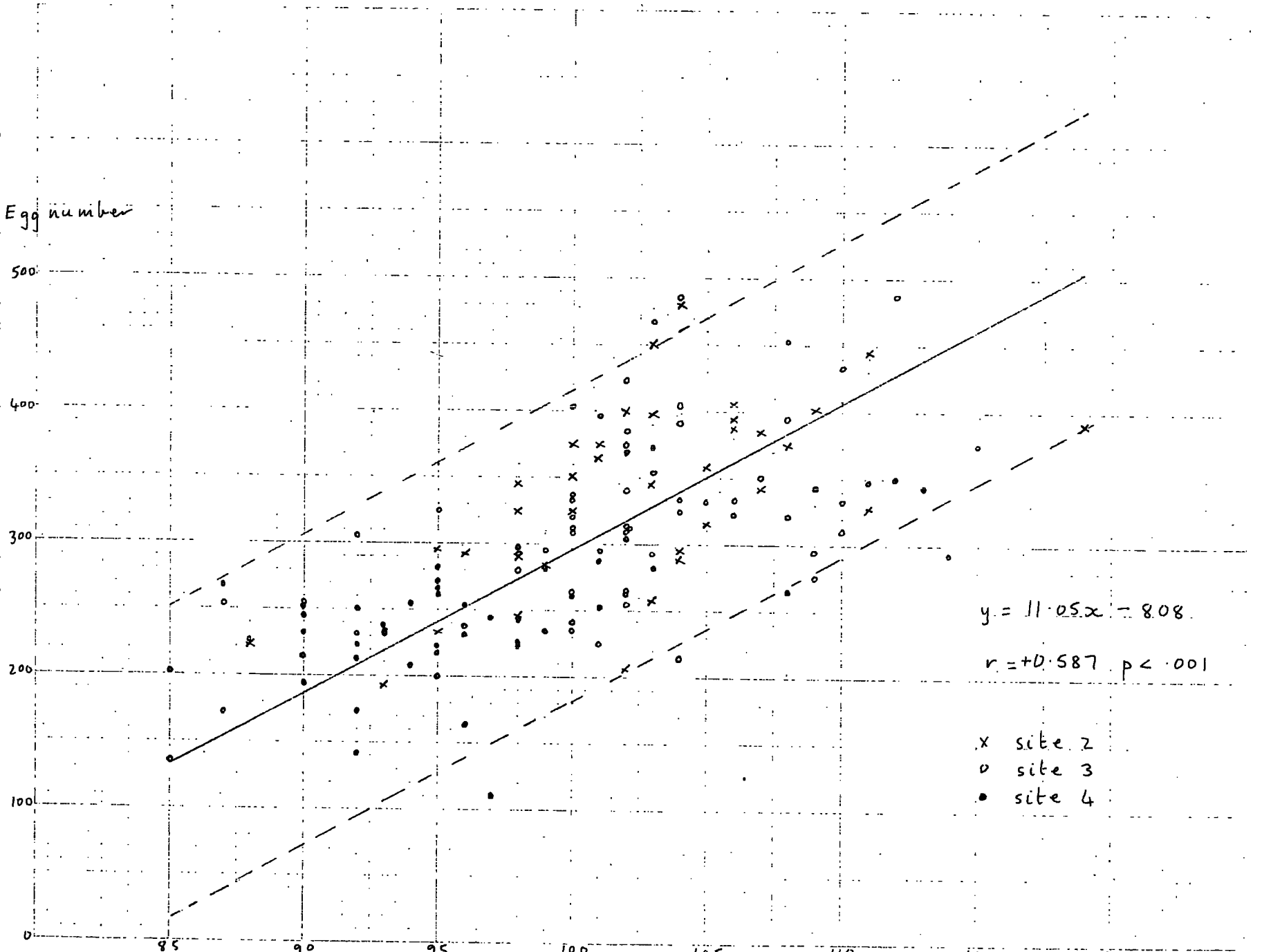


Fig.7 Egg number plotted against wing length

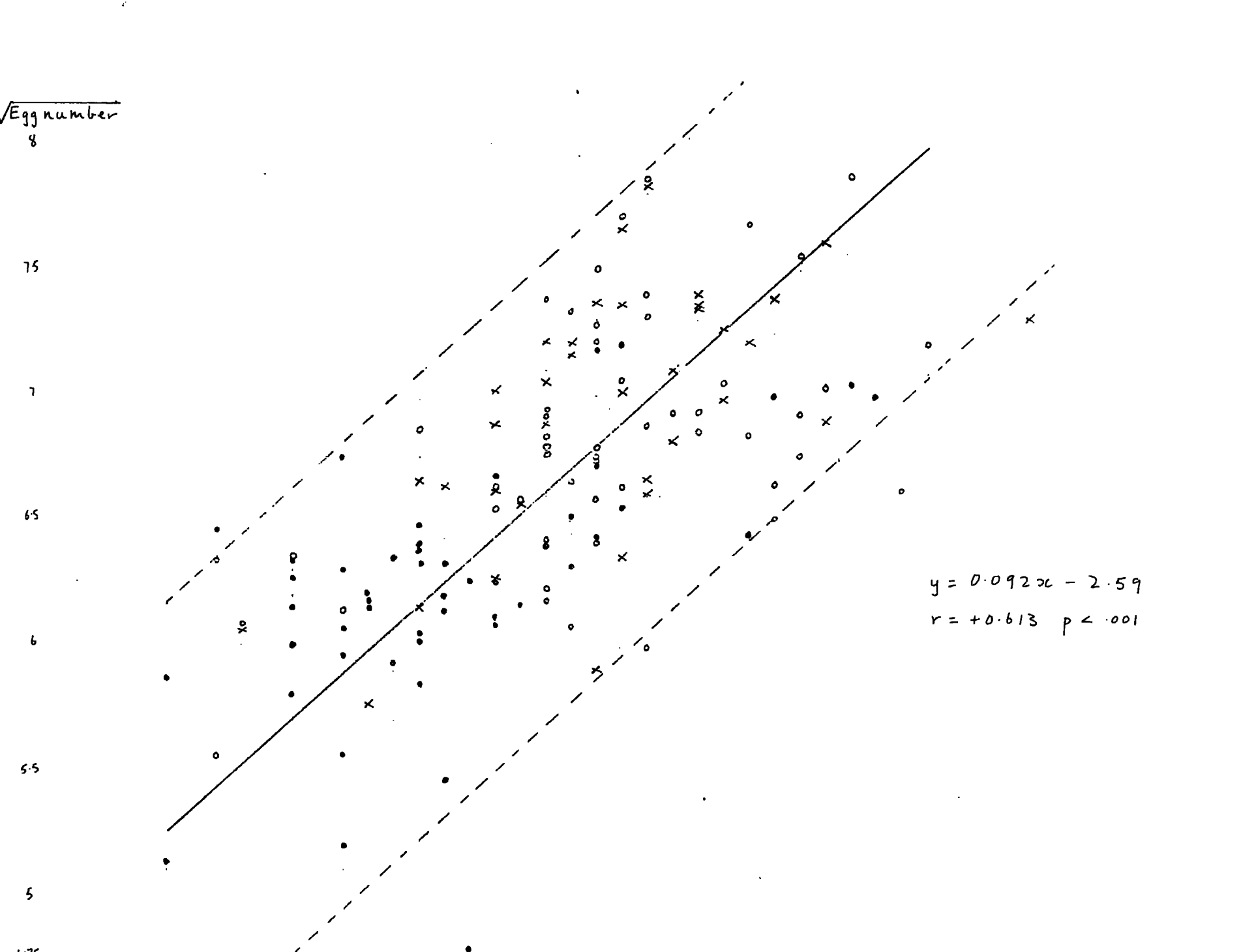


Fig. 8 $\sqrt{\text{Egg numbers}}$ plotted against wing lengths

Table 8. Showing the variation in mean wing length throughout emergence

Dates	Site 1			Site 2			Site 3			Site 4		
	Mean wing length	SE	N+	Mean wing length	SE	N+	Mean wing length	SE	N+	Mean wing length	SE	N
May 23-25	-	-	-	-	-	-	-	-	-	96.9	±0.63	75
26-28	-	-	-	-	-	-	-	-	-	97.1	±0.58	154
29-31	102.1	±0.69	98	106.8	±0.98	46	103.5	±0.61	108	97.4	±0.42	150
June 1- 3	103.2	±0.64	92	102.9	±0.58	115	102.7	±0.57	135	97.3	±0.58	150
4- 6	102.8	±0.62	149	102.0	±0.62	142	104.7	±0.57	150	97.5	±0.63	119
7- 9	101.5	±0.63	146	101.8	±0.65	149	104.7	±0.87	60	95.8	±1.13	50
10-12	97.5	±1.75	15	99.5	±0.73	115	-	-	-	-	-	-

+N = number in the sample

Table 9. Showing variation in mean wing length throughout the emergence. The significance of the changes in value from period to period is compared using student's t-test

Dates	Mean wing length	^{+}N	t	p
May 23 - 25	96.9	75		
			0.235	not sig
25 - 28	97.1	154		
			4.23	$<.001$
29 - 31	101.2	402		
			0	not sig
June 1 - 3	101.2	491		
			2.8	$<.01$
4 - 6	102.0	558		
			1.22	not sig
7 - 9	101.4	405		
			2.7	$<.01$
10 - 11	99.3	130		

^{+}N = number in sample

Table 7. Showing a comparison between means of egg numbers derived by two different methods

	Sample in which the eggs were counted					Sample in which the egg numbers were derived from the regression				
Site	Sample size	Mean wing length	SE	Mean egg number	SE	Sample size	Mean wing length	SE	Mean egg number	SE
2	55	102.5	±0.81	326	±9.3	565	102.0	±0.33	315	±5.3
3	36	102.4	±0.95	338	±11.4	453	103.8	±0.32	340	±3.4
4	50	96.3	±0.78	248	±7.7	705	96.6	±0.35	250	±2.7

The mean wing length on each site was then used to calculate the mean egg numbers on each site using the relationship $y = 0.092 x - 2.59$ (where y is the cube root of the egg numbers). Table 7 shows a comparison between the means when the eggs were counted for each female and the means derived from the regression equation, for the three sites where female egg numbers were counted.

The variation in mean wing length from day to day

The variation in mean wing length from day to day on each site was grouped into 3 day periods to see whether the size of T.subnodicornis varied during different stages of the emergence period. These data are given in table 8. It can be seen from table 8 that the wing length appears to decrease during the last few days of emergence. This decrease is significant at the 5% level of probability for sites 1 and 2 but not for sites 3 and 4.

When the results for all the sites are pooled (table 9) it appears that the wing length mean is smaller at the beginning and end of emergence than during the middle. The decline in size at the end of emergence can be seen on individual sites and it would seem that late emergence is correlated with small size in T.subnodicornis. It is not clear whether early emerging flies tend to be small or whether the small size is related to the site where the emergence happens to be early.

Discussion of the relationship between egg number and adult density.

From the results of the present study and from the 1954 data it would appear possible that some density dependent factor was acting at the larval stage, operating on egg number so that on sites where the larvae were more numerous fewer eggs were produced. However this result may well be fortuitous. The correlation between adult density and the number of eggs produced depends on the validity of the density measurement. The two trapping methods used during the present study probably gave a poor indication of the density on site 5, mixed moor, relative to the other sites. On the other sites where the vegetation differences were small the different dates and duration of the emergence period on each site meant that the temperature changes affected the comparative trap results but this may have been compensated for by the use of the two methods as the two catches tended to counteract each other.

A further difficulty lies in the fact that any density dependent factor that is limiting egg numbers must be working on the larval stage. If the adult density does not reflect the larval density the correlation is not expected. Coulson (1956) found little mortality in the last stages of the fourth instar and pupae in 1954 but in this study 6 of the 13 larvae taken from site 1 at Netherhearth were parasitised by a black fungus which invaded the tracheal system. The larvae were easily identified by their blackened appearance. Coulson (1962)

found that infected larvae died within 7 days of introduction into the laboratory and it was found during this study that parasitised larvae died either before or during pupation. The percentage parasitisation found on site 1 (table 10) if spready throughout the site would have the effect of lowering the adult density considerably. Site 4 is also affected but not to such a great extent and it is impossible to predict what the percentage would be in a larger sample.

Table 10. Showing percentage of larvae parasitised at different sites

	Site 1	Site 2	Site 3	Site 4	Site 5
Number of larvae	13	7	5	7	13
Number parasitised	6	0	0	1	0
% of larvae parasitised	46	0	0	14	0

Despite doubts about the density estimation the correlation between mean egg numbers and the numbers of adults caught on traps (fig. 9) is negative and is significant at the 5% level. Coulson's figure for 1954 confirms the negative trend.

Further data are required and it would seem that the relationship between egg numbers produced on a site and the density of larvae on the site is worth investigation.

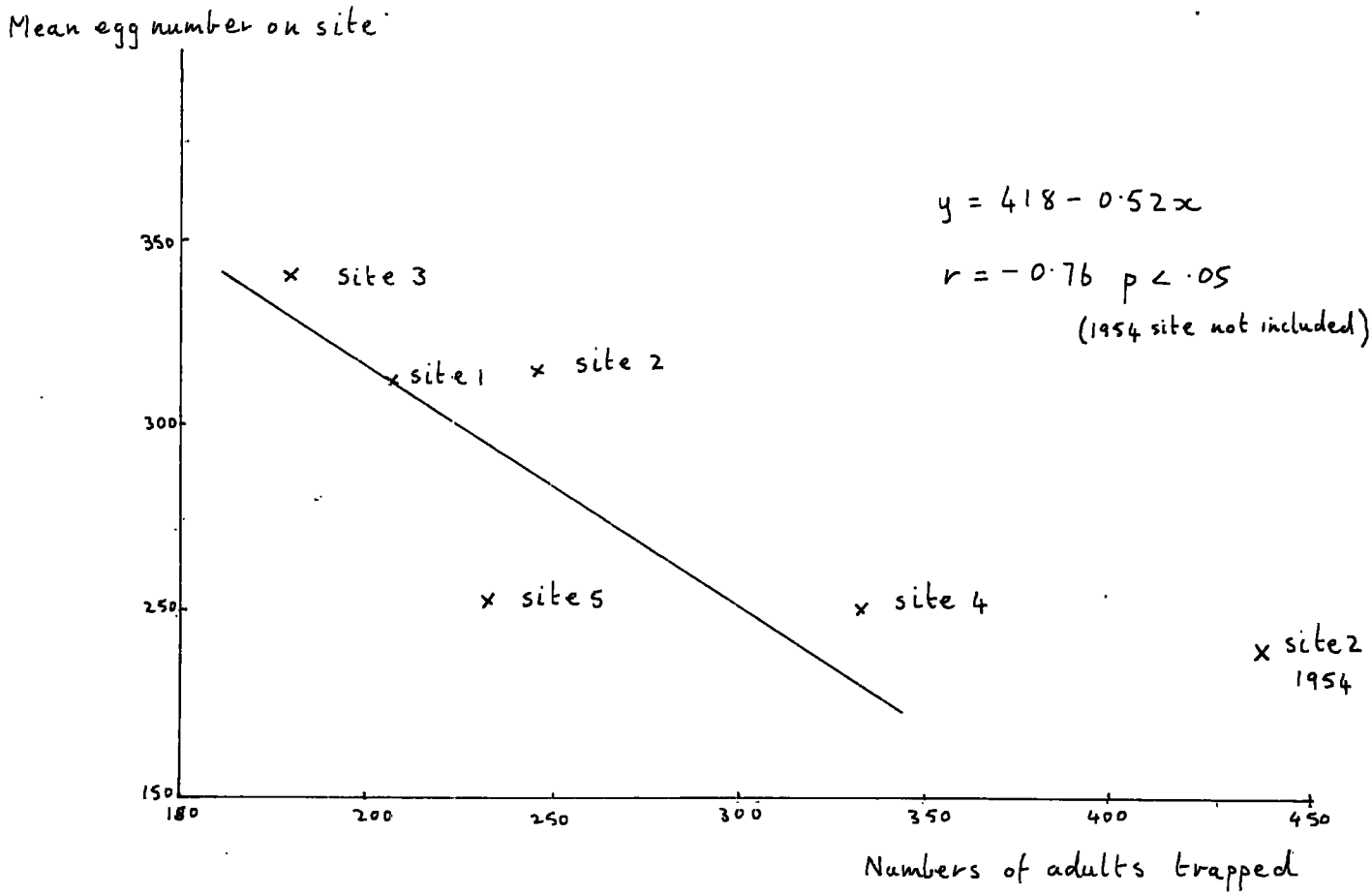


Fig. 9 Mean egg numbers (derived) for each site plotted against the numbers of adults trapped

DISCUSSION

Tipula subnodicornis is an ideal animal to study from the point of view of population regulation. The female is restricted in movement as an adult and presumably as a larva. It will deposit its eggs within a few metres of its place of emergence (Coulson 1956). The male has greater power of movement as an adult but this will have no effect on the relationship between the larval density in one year and the number of eggs carried by the females in that year. It would seem possible that, due to the limited powers of dispersal of the female, numbers on a site could build up from year to year until they were checked by the food supply running out.

Andrewartha and Birch (1954) envisaged a model in which the abundance of a species in a locality fluctuates with the random fluctuations of environmental factors, such as weather, which are normally considered as non density-dependent. The numbers in an area will depend on the rate of population increase and on the length of time the rate of increase in that part of the population is positive. When the rate of increase is reduced to zero by some catastrophe the part of the population concerned will become extinct. If the catastrophe has not been too far-reaching the area will be recolonised from nearby habitats which are for some reason better buffered against that particular catastrophe.

This type of situation was noticed by Coulson in 1955 at Moor House. The rainfall was so low during June,

July and August that the peat dried out and no larvae on Juncus squarrosus areas survived beyond the second instar. The numbers of final instar larvae found in sphagnum areas were not affected (Coulson 1956). His figures are shown in table II.

Table II Showing densities per square metre of final instar larvae from 1954-56 on three different types of vegetation (from Coulson 1956)

Habitat	1954	1955	1956
Juncus squarrosus	168	140	0
Eriophorum	70	104	4
Sphagnum Bog	17	36	14

He found that by 1962 the number of final instar larvae at Netherhearth on the Juncus area was back to its pre-drought level of 140 per square metre (Coulson 1962). Presumably the Juncus areas which under normal conditions support higher densities of larvae had been recolonised from the Sphagnum areas. The damper Sphagnum areas are usually less favourable but, during a drought, act as a reservoir when the major part of the population is destroyed.

If regulation in Nicholson's (1933, 1957) sense occurs it might operate in the periods between catastrophes. Either catastrophes occur at such frequent intervals that during favourable years the rate of increase never induces competition, or the rate of increase is such that numbers cannot be kept below the level of competition by periodic

elimination of parts of the population. If the first alternative operates it should be possible to relate fluctuations in density, on certain habitat types, to fluctuations in weather or some other non density dependent variable. In the case of the second alternative the relative stability of the density at a maximum value would be masked during most years by the effect due to random environmental fluctuation. The mortality each year would have to be analysed into its various components (Varley and Gradwell 1960, 1966) before any density dependent factors could be distinguished. Having made these reservations it is interesting to see from Coulson's data (table 12) that the larval densities on different types of vegetation are very similar in 1953 and 1954.

Table 12. Showing the larval (fourth instar) densities on four types of vegetation in two years (from Coulson 1956, 1962)

	1953			1954		
	Density/ m^2	No. of samples	No./ sample	Density/ m^2	No. of samples	No./ sample
Netherheath (Juncus area)	168	60	1.37	140	30	1.13
Eriophorum area	70	-	-	104	40	0.84
Valley Bog (Juncus area)	111	29	0.90	104	19	0.84
Valley Bog (Sphagnum area)	17	17	0.14	36	36	0.29

If Netherhearth alone is considered there is information for a further two years, 140 fourth instar larvae per square metre in 1962 and 40 per square metre in 1969. During 1953 and 1954 and again in 1962 the number of adult males seen flying appeared much greater than in intervening periods

and Coulson's impression (pers.comm.) and that of others living at Moor House is that since 1962 the densities have remained low.

The similar densities in 1953 and 1954 together with the impression that these were the highest observed over a number of years, indicate that at these levels there might be density dependent factors working against the natural rate of increase. Coulson (1962) found in 1954 that there was a 95% mortality in the egg and first instar stages. These two main phases of mortality reduced the density of T. subnodicornis at Netherhearth from 18000 eggs per square metre in June 1954 to 111 final instar larvae per square metre in May 1955. The mortality needs further analysis before either the causative factors or their effects on the density can be identified.

If density dependent factors do operate there are a number of ways in which regulation could be brought about. Competition for food or space could occur at all stages of the life-history. The accumulation of waste products increasing in concentration with increasing density of larvae could have toxic effects. Parasitism or disease would be passed more rapidly from individual to individual at higher densities. Predation might well be intensified in areas where densities were greatest.

Coulson (1962) found no evidence of parasitism at the egg stage but he found that 10% of the eggs were damaged by predators, presumably a larger percentage if those entirely destroyed could have been included. About 15% of the eggs were infertile or failed to develop. The

difference in the percentage hatching in 1954 and 1955 (78% and 72% respectively) was not significant but the percentage destroyed by predators, 4% in 1954 and 17% in 1955, was highly significant ($P < .01$) Predation on eggs could well have a density dependent effect. It might well be delayed (Varley and Gradwell,1966) with the numbers of the predator building up during a year when there are large numbers of eggs and providing a high density the following year.

There was no evidence to suggest that the high mortality in the first instar was due to anything other than severe weather conditions. The eggs and first instar larvae are very susceptible to drought and may be so to other non density dependent environmental factors. However it appeared during this study that the female laid several eggs in one cavity (judging from the number of times the abdomen was thrust downwards) and when there were female densities of 70 per square metre several females must have deposited eggs very close together. It is possible that early larvae develop less well when aggregated either because they compete for food or because their environment becomes contaminated.

At later stages in larval development the quality or quantity of food, which consists largely of the two liverworts Diplophyllum albicans and Ptilidium ciliare (Coulson 1956) might well become limiting. The accumulation of excretory products conditioning the area surrounding the larva might make temporary vacation of a site necessary. High larval densities would lead to

the majority of larvae not being able to escape contamination.

It is possible that even under favourable weather conditions such as those in 1953 and 1954 larvae can only survive in a very specialised micro-habitat and that these spaces are limited. If only the larvae in the centre of a Juncus plant, or some analogous situation, can survive the winter then the number of fourth instar larvae will be limited to the number of refuges and the ability of the larvae to find them.

Parasitism or disease of larvae could form a density controlling mechanism. Coulson (1962) found very little parasitism ; only 1 out of 200 larvae was found to be parasitised by an insect (Crocuta geniculata) and only 8 from 102 larvae were found to be suffering from the black fungus. The high proportion of larvae infected with the fungus found at site 1 during this study indicates that it can become an important mortality factor. It might well act in a density dependent way with a greater proportion of the insects at higher densities infecting each other.

Predation at the late larval stages is unlikely to be an important factor. Tipulid larvae form a high percentage of the diet of birds such as starlings (Dunnet, 1956) that visit arable land, but these birds are not present on moorland and the main vertebrate predator, the meadow pipit (Anthus pratensis) is absent from September to the end of March and has rarely been seen to take the larvae (Coulson 1962). Pupal mortality was not recorded except in the case of parasitised larvae

brought back to the laboratory.

The adults of T. subnodicornis form 75% of the food that the meadow pipit feeds to its young. This is however less than 1% of the available population of T. subnodicornis. Rana temporaria, spiders and a dipteran, Empis borealis, have also been recorded as preying on the adult stage (Coulson 1962) but it seems unlikely that they reduce the population to any greater extent than does the meadow pipit. The main hazard would appear to be the weather and as T. subnodicornis was seen copulating and ovipositing during the most severe weather encountered during this study it seems unlikely that mortality in the short adult stage will be very important.

The positive correlation between wing length and egg number established during this study indicates a correlation between size and egg numbers, so any density dependent factor that acts on growth will act on egg number. As the eggs are matured in the final instar larvae the factors most likely to affect the egg numbers are those inducing larval competition. Food shortage which is not severe enough to kill the larvae might restrict growth and egg development. The concentration of excretory products might also retard growth. Parasitism of final instar larvae is not likely to affect egg numbers in non parasitised females as the eggs are well developed before the parasitised larvae die.

Further studies need to be made on the mortality of T. subnodicornis so that the most important factors may be assessed. The greatest mortality is at the egg and first instar stages and there is at present no evidence to suggest that this is caused by

anything but non-density dependent environmental factors. However even if the early mortality accounts for 95% of the population, as it did in 1954, the 5% left would still allow a sevenfold increase from year to year, so the mortality in the late larval stages is of considerable importance.

The relative stability of the density on one site during three years indicated that there might be a density dependent factor operating. The larval food supply seemed likely to act as a regulating factor with the possibility of restriction in growth and egg development when supplies became inadequate. This study shows that egg numbers may decrease at high densities and that further investigation of the relationship between larval density and the mean numbers of eggs produced by females on a site would be worth while.

The correlation between wing length and egg numbers found during this study needs testing over a number of sites and in other years. If it proves valid it will be useful for assessing the egg numbers quickly on a large number of sites. It was shown during this study that during the peak of emergence on each site wing length did not differ significantly from one three day period to the next. In future years it should therefore be possible to collect samples from sites during the peak period of emergence, assessed from trap data, and estimate the mean number of eggs produced by females on each site. There will be no need to collect so thoroughly from each site nor to select teneral females. This will allow many more sites

to be used and the effects of different types of vegetation to be assessed. It should be possible to show a density dependent effect occurring at different levels of density on different vegetation types.

Summary

1. The comparative densities of T. subnodicornis on five sites were estimated by three methods of larval sampling, catching on sticky traps and in pitfalls and catching by hand.
2. The mean egg number produced by females on three sites was calculated from egg counts.
3. A correlation between egg numbers and wing length was found.
4. The relationship between egg numbers and wing length was used to estimate the mean egg numbers produced on all five sites.
5. A negative correlation significant at the 5% level was found between egg numbers produced on a site and the adult density on a site.

References

Andrewartha, H.G. 1957. The use of conceptual models in population ecology. Cold Spring Harb.Symp. Quant.Biol. 22, 219-234.

Andrewartha, H.G. & Birch, L.C. 1954. The distribution and abundance of animals. Chicago.

Birch, L.C. 1957. The role of weather in determining the distribution and abundance of animals. Cold Spring Harb.Symp. Quant.Biol. 22, 203-218.

Broadbent, L. 1948. Aphis migration and the efficiency of the trapping method. Ann.app.Biol. 35, 379-394.

Coulson, J.C. 1956. Biological studies on the meadow pipit (Anthus pratensis) and moorland Tipulidae; members of a food chain. Thesis : Durham University Library.

Coulson, J.C. 1959. Observations on the Tipulidae (Diptera) of the Moorhouse Nature Reserve, Westmorland. Trans.R.Ent.Soc. Lond. 111, 157-174.

Coulson, J.C. 1962. The Biology of Tipula subnodicornis Zetterstedt, with comparative observations on Tipula paludosa Meigen. J.Anim.Ecol. 31, 1-21.

Dunnet, G.M. 1956. The autumn and winter mortality of Starlings Sturnus vulgaris in relation to their food supply. Ibis, 98, 220-230.

Greenslade, P.J.M. 1964. Pitfall trapping as a method for studying populations of Carabidae (Coleoptera). J.Anim.Ecol. 1964, 301-310.

Hadley, M.J. 1966. Biological studies on Molophilus ater Meigen (Diptera : Tipulidae). Thesis : Durham University Library.

Manley, G. 1936. The climate of the northern Pennines : the coldest part of England. Quant.J.R.met.Soc. 62, 103-115.

Manley, G. 1943. Further climatological averages for the northern Pennines, with a note on topographical effects. Ibid. 69. 251-262.

Mather, K. 1949. Statistical analysis in Biology. London.

Nicholson, A.J. 1933. The balance of animal populations. J.Anim.Ecol. 2 : (Suppl.) 132-178.

Nicholson, A.J. 1957. The Self-adjustment of Populations to change. Cold Spring Harb.Symp.quant.Biol. 22, 153-171.

Pearsall, W.H. 1950. Mountains and Moorlands. London.

Taylor, L.R. 1963. Analysis of the effect of temperature on insects in flight. J.Anim.Ecol. 32, 99-117.

Varley, G.C. & Gradwell, G.R. 1960. Key factors in population studies. J.Anim.Ecol. 29, 399-401.

Varley, G.C. & Gradwell, G.R. 1968. Population models for the Winter Moth. Roy.Ent.Soc.Symp. 4, 132-142.

Welch, D. 1964. Studies in the autecology of Juncus squarrosus L. Thesis : Durham University Library.